YieldAce Hotstart DNA Polymerase

INSTRUCTION MANUAL

Catalog #600334 (1000 U) and #600336 (3000 U)
Revision A.01

For In Vitro Use Only
600330-12

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MATERIALS PROVIDED

Materials provided	Catalog #600334 (1000 U)	Catalog #600336 (3000 U)
YieldAce hotstart DNA polymerase (5 U/µl)	200 µl	3 × 200 µl
10× YieldAce reaction buffer	4 × 1 ml	12 × 1 ml

STORAGE CONDITIONS

All Components: –20°C

NOTICES TO PURCHASER

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Revision A.01

INTRODUCTION

YieldAce Hotstart DNA Polymerase* is a unique enzyme formulation specifically developed for maximizing PCR product yields while providing hotstart capabilities.[1] YieldAce Hotstart DNA polymerase is formulated with heat labile monoclonal antibodies that, at room temperature, effectively neutralize DNA polymerase activity. Full enzyme activity is regained upon denaturation of the antibody during the initial denaturation step. The enzyme and PCR reaction buffer have been optimized to provide superior performance in a variety of applications routinely carried out with *Taq* DNA polymerase. Since YieldAce DNA polymerase differs significantly from *Taq* DNA polymerase, it is important to follow the recommended protocol for optimal performance. Compared to *Taq* DNA polymerase, YieldAce DNA polymerase synthesizes higher yields of product from a variety of DNA templates, including plasmid DNA, genomic DNA, and cDNA, and it readily amplifies templates directly from bacterial cultures. Additionally, reactions performed with YieldAce DNA polymerase show less variation in product yield, with respect to amplicon size and DNA template concentration, than identical reactions performed with *Taq* DNA polymerase.

A key component of YieldAce hotstart DNA polymerase is the ArchaeMaxx polymerase-enhancing factor. The ArchaeMaxx factor eliminates a PCR inhibitor and promotes shorter extension times, higher yield, and greater target length capabilities. The ArchaeMaxx factor improves the yield of products by overcoming dUTP poisoning, which is caused by dUTP accumulation during PCR through dCTP deamination.[2] Once incorporated, dU-containing DNA inhibits *Pfu* and most archaeal proofreading DNA polymerases, such as Vent® and Deep Vent® DNA polymerases, limiting their efficiency.[2] The ArchaeMaxx factor functions as a dUTPase, converting poisonous dUTP to harmless dUMP and inorganic pyrophosphate, resulting in improved overall PCR performance.

* U.S. Patent Nos. 6,734,293; 6,489,150, 6,444,428, 6,183,997, 5,948,663, 5,866,395, 5,556,772, 5,545,552 and patents pending.

CRITICAL OPTIMIZATION PARAMETERS

All PCR amplification reactions require optimization to achieve the highest product yield and specificity. Critical optimization parameters for successful PCR using YieldAce hotstart DNA polymerase are outlined in Table I and discussed in the following section. These include the quality and concentration of DNA template, the appropriate concentration of dNTPs, sufficient enzyme concentration, optimal cycling parameters, and appropriate PCR primer design. The magnesium chloride supplied in the 10× reaction buffer is optimized for YieldAce hotstart DNA polymerase and most targets. Further optimization is rarely necessary.

TABLE I

Optimization Parameters and Suggested Reaction Conditions

Parameter	Suggested Condition (50 µl reaction volume)
Input template	100–250 ng genomic DNA 1–30 ng vector DNA 0.5–2 µl bacterial culture 1–4 µl cDNA synthesis reaction
YieldAce hotstart DNA polymerase	2.5 U
dNTP concentration	200 µM each dNTP (0.8 mM total)
Primers (each)	100 ng (0.25 µM)
Extension time	1 min per kb
Denaturing temperature	95°C
Extension temperature	72°C

DNA Template

Template Quality

Potential shearing of the genomic DNA template is minimized by the use of wide-bore tips for pipetting or mixing of the template. Freezing of high molecular weight templates should be avoided; storage at 4°C is recommended. Bacterial cultures may be directly amplified from glycerol stocks or fresh overnight cultures.

Template Concentration

When amplifying genomic DNA templates, use 100–250 ng of template for reaction volumes of 50 µl. To amplify low-complexity targets (for example, lambda or plasmid DNA), use 1–30 ng of template or 0.5–2 µl of a bacterial culture. To amplify cDNA, 1–4 µl of a standard cDNA reaction is recommended.

Enzyme Concentration

Robust product yield requires an adequate DNA polymerase concentration. The use of 2.5 U/50-µl reaction consistently generates high yield from most templates. Higher concentrations of enzyme in the reaction mixture can be inhibitory.

Deoxynucleotide Concentrations

Amplification efficiencies are influenced by deoxynucleotide (dNTP) concentrations. Insufficient concentrations of dNTPs may result in lower yields. We recommend using each dNTP at a final concentration of 200 µM.

Primer Design and Concentration

Primers should be ≥23 bp in length with a balanced T_m ≥60°C. The resulting high annealing temperature promotes specificity and discourages secondary structure formation. Further, primer sequences should be analyzed for potential duplex and hairpin formation as well as false priming sites in order to obtain the highest yield of specific PCR products.

We recommend using ~0.25 µM final concentration of each primer. When using 25-mer oligonucleotide primers in a 50-µl reaction volume, this is equivalent to ~100 ng of each primer.

Cycling Parameters

As with all PCR reactions, cycling parameters are critical for successful amplification and may require further optimization.

Extension Time

Maintain an extension time of 1.0 minute (for targets ≤1 kb) or 1.0 minute/kb of template (>1 kb targets) for general applications; longer extension times may produce higher yields.

Extension Temperature

Extension temperatures also have a critical effect on amplicon yield. An extension temperature of 72°C should be used with all templates.

Denaturation Temperature

High denaturation temperatures damage DNA templates, so the denaturation temperature should be as low as possible. A denaturation temperature of 95°C works well for most targets.

PROTOCOL

1. Prepare a reaction mixture for the appropriate number of samples to be amplified. The following table provides an example of reaction mixtures for the amplification of genomic and plasmid DNA targets. The recipe listed in the table is for one reaction and can be scaled for multiple samples. Add the components *in order* and mix gently.

Component	Quantity per reaction	
	Genomic targets	**Plasmid targets**
Distilled water	*X* µl to final volume of 50.0 µl	*X* µl to final volume of 50.0 µl
10× YieldAce reaction buffer	5.0 µl	5.0 µl
dNTP mix (25 mM of each dNTP)	0.4 µl	0.4 µl
DNA template: Genomic DNA Plasmid DNA	 100–250 ng* —	 — 1–30 ng* or 0.5–2 µl bacterial culture
Primer #1	100 ng	100 ng
Primer #2	100 ng	100 ng
YieldAce hotstart DNA polymerase (5 U/µl)	0.5 µl	0.5 µl
Total reaction volume	50.0 µl	50.0 µl

* Excess template can be inhibitory to the PCR reaction. Do not exceed the recommended quantities.

2. Before thermal cycling, aliquot 50 µl of the master mixture into sterile thin-walled PCR tubes.

3. If the temperature cycler is not equipped with a heated cover, overlay each reaction with ~50 µl of DNase-, RNase-, and protease-free mineral oil (available from Sigma Chemical Company, St. Louis, Missouri).

4. Perform PCR using optimized cycling conditions. Suggested cycling parameters are given below for (A) single-block temperature cyclers and (B) RoboCycler temperature cyclers:

(A)

Single-Block Temperature Cyclers

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	10	95°C	20 seconds
		Primer T_m – 5°C[a]	20 seconds
		72°C	60 seconds/kb of PCR target[b]
3	20	95°C	20 seconds
		Primer T_m – 8°C[a]	20 seconds
		72°C	60 seconds/kb of PCR target plus 10 seconds/cycle[b]
4	1	72°C	7 minutes

[a] The annealing temperature may be lowered or raised if necessary to obtain optimal results.

[b] The minimum extension time is 1 minute.

(B)

RoboCycler Temperature Cyclers

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	10	95°C	50 seconds
		Primer T_m – 5°C[a]	50 seconds
		72°C	90 sec./kb[b]
3	20–25	95°C	50 seconds
		Primer T_m – 8°C[a]	50 seconds
		72°C	90 sec./kb[b]
4	1	72°C[b]	7 minutes

[a] The annealing temperature may be lowered or raised if necessary to obtain optimal results.

5. Analyze the PCR amplification products by electrophoresis using an appropriate percentage acrylamide or agarose gel.

TROUBLESHOOTING

Observations	Suggestions
No PCR product or lower yield than expected	Verify the concentration of DNA template. Titrate the amount of full-length intact DNA template and/or increase the number of cycles up to a maximum of 40 cycles
	Use intact and highly purified DNA templates
	Store the template at 4°C; do not freeze the template
	Lower the annealing temperature in 5°C increments
	Allow at least 60 seconds of extension time for each kilobase to be amplified (90 seconds of extension time per kilobase may also be helpful for difficult templates)
	Denaturation times of 50 seconds for RoboCycler temperature cyclers or 20 seconds for single-block temperature cyclers at 95°C are usually sufficient, longer denaturation times or higher denaturation temperatures may damage the DNA template; use the shortest denaturation time compatible with successful PCR on the thermal cycler
	Primer pairs exhibiting matched primer melting temperatures (T_m) and complete complementarity to the template are recommended
	Analyze the primer sequences to ensure that duplexes or hairpins do not form
	Gel-purified or HPLC-purified primers ≥23 nucleotides in length are desired for successful PCR
	Adjust the ratio of primer versus template to optimize yield of the desired product
	Use thin-wall PCR tubes for Stratagene thermal cyclers. These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance
	Use DMSO in the PCR mixture; titrate the DMSO concentration in 1% increments
Artifactual PCR smears	Decrease the amount of YieldAce Hotstart polymerase
	Reduce the extension time
	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used
Multiple bands	Increase the annealing temperature in 3°C increments in segment 2
	Use Perfect Match PCR enhancer to improve PCR product specificity
	Use DMSO in the PCR mixture; titrate the DMSO concentration in 1% increments
	Verify that the primers hybridize only to the desired sequences on the template

REFERENCES

1. Borns, M. and Hogrefe, H. H. (2000) *Strategies* 13(4):125–127.
2. Hogrefe, H. H., Hansen, C. J., Scott, B. R. and Nielson, K. B. (2002) *Proc Natl Acad Sci U S A* 99(2):596-601.
3. Borns, M., Scott, B. and Hogrefe, H. H. (2001) *Strategies* 14(1):5–8.
4. Eads, J., Bai, F., Allen, R. and Hogrefe, H. H. (2000) *Strategies* 13(4):138–140.

ENDNOTES

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.